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A fantastic new species of secretive forest frog discovered from forest fragments near Andasibe, Madagascar

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http://zoobank.org/4DC62CDB-A6FB-472D-9E6D-FDBF4E101785

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Abstract

We describe a fantastic new species of forest frog (Mantellidae: *Gephyromantis*: subgenus: *Laurentomantis*) from moderately high elevations in the vicinity of Andasibe, Madagascar. This region has been surveyed extensively and has a remarkably high anuran diversity with many undocumented species still being discovered. Surprisingly, by exploring areas around Andasibe that lacked biodiversity surveys, we discovered a spectacular and clearly morphologically distinct species, previously unknown to science, *Gephyromantis marokoroko* **sp. nov.**, documented for the first time in 2015. The new species is well characterised by a very rugose and granular dorsum, dark brown skin with bright red mottling, sparse light orange to white spots on the ventre, vibrant red eyes and femoral glands present only in males that consist of eight medium-sized granules. Bioacoustically, the new species has a quiet advertisement call that differs from related species by having a moderate call duration, 2–4 strongly pulsed notes and a slow note repetition rate. Furthermore, it has substantial differentiation in mitochondrial DNA, with pairwise distances of 7–9% to all other related species in sequences of the mitochondrial 16S rRNA marker. Additional evidence is given through a combined four mitochondrial markers and four nuclear exons concatenated species tree, strongly supporting *G. striatus* as the sister species of the new species in both analyses. The discovery of this new species highlights the need for continued inventory work in high elevation rainforests of Madagascar, even in relatively well-studied regions.

Key Words

Amphibia, Anura, Andasibe, *Gephyromantis marokoroko*, Mantadia, new species, taxonomy

Introduction

Madagascar hosts an impressively diverse and almost entirely endemic amphibian fauna, diversified into a multitude of different habitats and micro-habitats (Glaw and Vences 2007). Amongst the small, brown leaf litter frogs, members of the genus *Gephyromantis* Methuen, 1920 are well represented in Madagascar. Most small, brown, leaf litter frogs in Madagascar are members of

Microhylidae, but Mantellidae has some representatives in the genus *Gephyromantis* Methuen, 1920. *Gephyromantis* is a genus within the Malagasy-Comoran endemic family Mantellidae (Glaw and Vences 2007). Within *Gephyromantis*, there are 47 recognised species belonging to six subgenera (Glaw and Vences 2006; Vences et al. 2017; AmphibiaWeb 2021), which are supported by molecular and morphological criteria (Glaw and Vences 2006; Wollenberg et al. 2011; Kaffenberger et al. 2012).

Recently, Madagascar's unique biota has been the focus of intensive field surveys (e.g. Goodman and Benstead 2003; D'Cruze et al. 2009; Hutter et al. 2015; Scherz et al. 2017a), with many previously unknown species discovered, typically through extensive molecular identification of candidate species lineages (Vieites et al. 2009; Perl et al. 2014). This is true for *Gephyromantis*, which has several candidate lineages identified (Scherz et al. 2017a, 2017b; Vences et al. 2017), some of which have recently been described (Scherz et al. 2017b, 2018). Despite these barcoding efforts, entirely new species, not previously identified from molecular barcoding, are still being discovered and described (Scherz et al. 2017a; Scherz et al. 2018), suggesting that many new species to science remain elusive.

Herein, we describe another *Gephyromantis* species new to science from the subgenus *Laurentomantis* that has not been previously identified through molecular barcoding studies. This new species is not morphologically cryptic and was immediately recognisable as a new species upon discovery in recent expeditions to remote high-elevation forests surrounding the village of Andasibe that lack biodiversity surveys. Despite the Andasibe region being one of the most well-studied areas in Madagascar (Vieites et al. 2009), this study shows that clearly recognisable new species that have not been barcoded are still being discovered by recent surveys. As a result of these discoveries, we emphasise that continued exploration and surveys in Madagascar are needed, even in relatively well-studied regions. Conservation of small forest fragments is especially important, as many undiscovered species may remain undetected.

Materials and methods

Terminology

We follow the unified concept of species (i.e. general lineage concept), which defines a species as a separately evolving lineage (Simpson 1961; Wiley 1978; de Queiroz 1998, 2005, 2007). We use multiple lines of evidence (i.e. secondary criteria) in assessing species boundaries, combining data from morphology, phylogenetics, bioacoustics and biogeography (de Queiroz 2007; Padial et al. 2010; Vences et al. 2013). This evidence is then considered equally and used as support for the hypothesis that a given population is an independently evolving lineage and, thus, a distinct species. Family and generic names follow the taxonomy proposed by Glaw and Vences (2006). Geographic regions for biogeographic analyses are defined according to Boumans et al. (2007). According to this zonation, the Andasibe area is within a region named the "Northern Central East" of Madagascar.

Specimen collection and morphological measurement

Specimens were collected at night through targeted searches of the new species' microhabitat. Specimens were euthanised using Tricaine, fixed in ~ 10% formalin (buffered with sodium phosphate to ~ 7.0 pH) for 24 hours and then stored in 70% ethanol for long-term preservation. We deposited and examined alcohol-preserved specimens from the amphibian collections at the Biodiversity Institute of the University of Kansas (KU) and Département de Biologie Animale, Antananarivo (UADBA) (Appendix I). Additional collection acronyms used herein are FAZC, ZCMV, FGZC and LR (field number series of F. Andreone, M. Vences, F. Glaw and L. Raharivololoniaina, respectively), FGMV (field number series shared between M. Vences and F. Glaw) and ZSM (Zoologische Staatssammlung München, Germany). All photographs were taken by CRH, unless otherwise noted.

Morphological measurements were taken by ZFA with a Mituyo digital caliper (precision 0.01 mm) rounded to 0.1 mm. Terminology and measurements largely follow Glaw et al. (2001) and we used the following: (1) snout-vent length (SVL); (2) head width at the greatest point (HW); (3) head length (= rostrum) from snout tip to posterior edge of tympanum (HL); (4) horizontal eye diameter (ED); (5) interorbital distance (IOD); (6) eye-snout tip distance (ESD); (7) eye-nostril distance (END); (8) distance from nostril-snout tip (NSD); (9) distance between nostrils (NND); (10) horizontal tympanum diameter (TD); (11) upper arm length (humerus), from the articulation of the arm with the trunk to the elbow (UAL); (12) lower arm length (= radioulna), from the humerus-radioulna articulation point (elbow) to carpal-metacarpal articulation (LAL); (13) hand length from carpal-metacarpal articulation to tip of longest finger (HAL); (14) forelimb length, sum of UAL, LAL and HAL (FORL); (15) forearm length, summed from and UAL and LAL (FARL); (16) Finger I length from outer margin of palmar tubercle to tip of Finger I (FIL); (17) Finger II length from outer margin of palmar tubercle to tip of Finger II (FIIL); (18) femur length from femur-tibia articulation (knee) to cloaca (FEML); (19) tibia length from femur-tibia articulation to heel, measured along the shank (TIBL); (20) tarsus length from heel to base of foot (TARL); (21) foot length from tarsal-metatarsal articulation to tip of longest toe (FOL); (22) length of femoral gland, horizontal across the thigh (FGL); (23) width of femoral gland (FGW); and (24) the number of femoral gland clusters on each thigh (FGC).

DNA sequencing and phylogenetics

Following euthanasia, we extracted whole livers and left hind limb muscles and stored the tissues in 95% ethanol. We obtained new genetic data for four specimens of the new species and one specimen from five other species in *Laurentomantis* from the 3' fragment of the 16S rRNA mitochondrial marker widely used for molecular comparisons and species barcoding in Mantellidae (e.g. Vieites et al. 2009). The methods for DNA extraction, primers used, PCR amplification and sequencing are described

in Hutter et al. (2018). Finally, additional mitochondrial and nuclear markers from one specimen of the new species (KU 343230) were acquired by extracting the target markers from samples sequenced using the FrogCap Ranoidea-V1 probe-set (Hutter et al. 2021; available at: https://github.com/chutter/FrogCap-Sequence-Capture). Probe design, sequencing and analytical methods are described in Hutter et al. (2021) in detail. After sequencing, DNA data were manually edited for quality in Geneious R9 (Biomatters 2016). Sequences were deposited in GenBank and their associated voucher specimens and accession numbers are provided in Appendix I.

We aligned the new sequences with 16S sequences from Kaffenberger et al. (2012) to confirm the subgeneric relationship of the new species (tree not shown). We next chose sequences for 16S from all *Laurentomantis* and several representatives from other species in *Gephyromantis* and the distantly-related *Mantella madagascariensis* as outgroups. In total, we supplemented these new data with 182 published sequences of *Gephyromantis* specimens from GenBank. The distantly-related *Mantella madagascariensis* was used as an outgroup to root the phylogeny. GenBank accession numbers and their associated specimen data are included in Appendix I.

The 16S rRNA sequence data were first aligned with MAFFT v.7.3 using the RNA alignment algorithm Q-ins-I (Katoh and Stanley 2013). We used Maximum Likelihood (ML) in IQ-Tree v.1.5.5 (Nguyen et al. 2015) to conduct phylogenetic tree reconstruction with default options selected. We used ModelFinder (Kalyaanamoorthy et al. 2017) to find a best-fit partitioning scheme and selected models of molecular evolution for each partition considering all models. We assessed support using 1000 ultrafast bootstrap replicates (Minh et al. 2013). Strongly supported nodes are those with 95 or higher bootstrap (BS).

For Bayesian Inference (BI), we used MrBayes 3.2 (Ronquist et al. 2012) and the best partitions and models selected above. We used reversible jump Markov Chain Monte Carlo to accommodate uncertainty in model selection (parameter set: *nst=mixed*). The analysis was run for two independent runs of 50 million generations sampling every 1000 generations. Chain mixing and stationarity were assessed by examining the standard deviation of split frequencies and by plotting the -lnL per generation using Tracer 1.5 software (Rambaut and Drummond 2007), where we discarded 25% of the generations as burn-in. Finally, results were combined using logCombiner 1.10 software (Rambaut and Drummond 2007) to obtain a 50% majority rule consensus tree and node posterior probabilities. Strongly supported nodes are those with a posterior probability (PP) of 0.95 or higher.

Bioacoustics

Advertisement calls were recorded in the field with a Marantz PMD 661 MKII Field Recorder and a Sennheiser MKH 8060 shotgun microphone. The calls were recorded in WAV format with a sampling rate of 44.1 kHz/s with 16 bits/sample. Advertisement calls analysed here have been deposited on FigShare (10.6084/m9.figshare.16728994). Calling males were recorded while inside plastic collecting bags at ~ 100 cm because we could not approach them close enough to record them in the field (we did not perceive a difference between the captive and *in situ* advertisement calls). We measured call parameters using RavenPro 1.5 (K. Lisa Yang Center for Conservation Bioacoustics 2014). Frequency information was obtained through Fast Fourier Transformation (FFT; width 1012 points). A Hanning window (512 bands) was used to create the spectrogram. Measures are reported as the range followed by the mean ± two standard deviations from the mean. Terminology generally follows Köhler et al. (2017), with a call defined as the entire assemblage of acoustic signals emitted in sequence and notes are subunits separated by temporally distinct segments of background noise between each note.

We chose the following relevant call variables, generally following the call-centred definitions of Köhler et al. (2017) and Hutter et al. (2013; Table 1): (1) number of notes per call; (2) call duration (ms); (3) call interval duration (ms); (4) note duration (ms); (5) inter-note interval duration (ms); (6) note repetition rate within call (notes/s); (7) pulse rate (/s); (8) dominant frequency, measured at peak amplitude (Hz); and (9) frequency bandwidth (Hz), measured as 90% of the sound energy.

Finally, we evaluated the amount of bioacoustic differences between species following Vieites et al. (2009). We considered differences in general call structure (e.g. pulsed/tonal notes, consistent note arrangements, amplitude envelope shape; Ryan and Rand 1990) and such temporal variables that are putatively less influenced by temperature, body size and behaviour (e.g. note duration, pulse rate; Gerhardt et al. 2000) to be important traits for distinguishing species.

Results

We discovered a morphologically distinct new species belonging to the subgenus *Laurentomantis* from *Gephyromantis* in the Andasibe area of Northern Central East Madagascar (Fig. 1), present at high elevations in several small forest fragments. The new species can be readily identified morphologically through its rugose and granular dorsal texture with prominent ridge elements, red dorsal colouration on a dark brown background, bright red eyes, the relatively large number of eight granules within each femoral gland and absence (or indistinction) of vertebral stripe (Fig. 2). Finally, comparisons of the uncorrected raw genetic distances give a minimum distance of 6–9% with *G. ventrimaculatus* in the mitochondrial marker 16S rRNA and greater distances with other species in *Laurentomantis* (Fig. 3). Phylogenetically, the new species position is poorly supported in 16S rRNA, but strongly supported sister to *G. striatus* in the combined nine marker mitochondrial and nuclear phylogeny (BS = 98; PP = 1.00;

Fig. 4). Furthermore, the genetic distances between *G. striatus* and the new species are 7–9%. The new species also has an advertisement call similar to that of other *Laurentomantis*, but can be distinguished through the combination of a moderate call duration, differing note structure with 2–4 clearly defined pulses and slower note repetition rate when compared to related species. Given the strong evidence, we describe the new species as follows:

Gephyromantis marokoroko sp. nov.

http://zoobank.org/3A22A655-D3B9-4C69-BF6F-C8F1E9595D84

Common English name: The Rugose Forest Frog.

Common Malagasy name: Ny sahon'ala marokoroko.

Holotype. KU 343230 (field number CRH 1110), an adult male collected by Carl R. Hutter, Shea M. Lambert and Zo F. Andriampenomanana collected on 5 January 2016, at Vohidrazana Forest (18.976°S, 48.499°E; ca. 1150 m a.s.l.) in mid-altitude rainforest near Andasibe in Northern Central East Madagascar (Fig. 1).

Paratypes. Adult male KU 343229 (CRH 1108), adult female UADBA-CRH 472 and adult male UADBA-CRH 1061 collected on 5 January 2016, with same collection data as holotype. Adult female KU 343218 (CRH 1397) collected on 18 January 2016, at Tavolobe (19.005°S, 48.461°E; ca. 1100 m a.s.l.) by Carl R. Hutter, Shea M.

Lambert, Ginah Tsiorisoa Andrianasolo and Kerry A. Cobb. Adult male UADBA-CRH 1626, Adult female UADBA-CRH 1819 collected on 6 January 2017 at Vohidrazana Forest, adult female KU 347328 (CRH 1923) collected on 14 January 2017 at Andasibe-Mantadia National Park (Belakato: 18.821°S, 48.439°E; ca. 1050 m a.s.l.) and adult female KU 347329 (CRH 2019) collected on 21 January 2017 at Vohimana (18.926°S, 48.489°E; ca. 1050 m a.s.l.), collected by Carl R. Hutter, Jary Harinarivo and Robin K. Abraham.

Available names. There are no junior synonyms available that could be assigned to the new species from the subgenus *Laurentomantis*.

Etymology. The specific epithet *marokoroko* is a Malagasy word meaning "rugose" or "rugged". The name was chosen to describe the rugose skin texture of this species. The name is to be treated as an invariable noun in apposition.

Diagnosis. *Gephyromantis marokoroko* (Fig. 2) is a member of the family Mantellidae, subfamily Mantellinae, as diagnosed by Glaw and Vences (2006). The new species can be diagnosed to the genus *Gephyromantis* morphologically through its granular dorsum, moderately enlarged fingertips, absence of foot webbing, bifid tongue and small femoral glands present only in males as a small number of large granules (type 2; Glaw et al. 2000). Within *Gephyromantis*, the new species can be diagnosed to
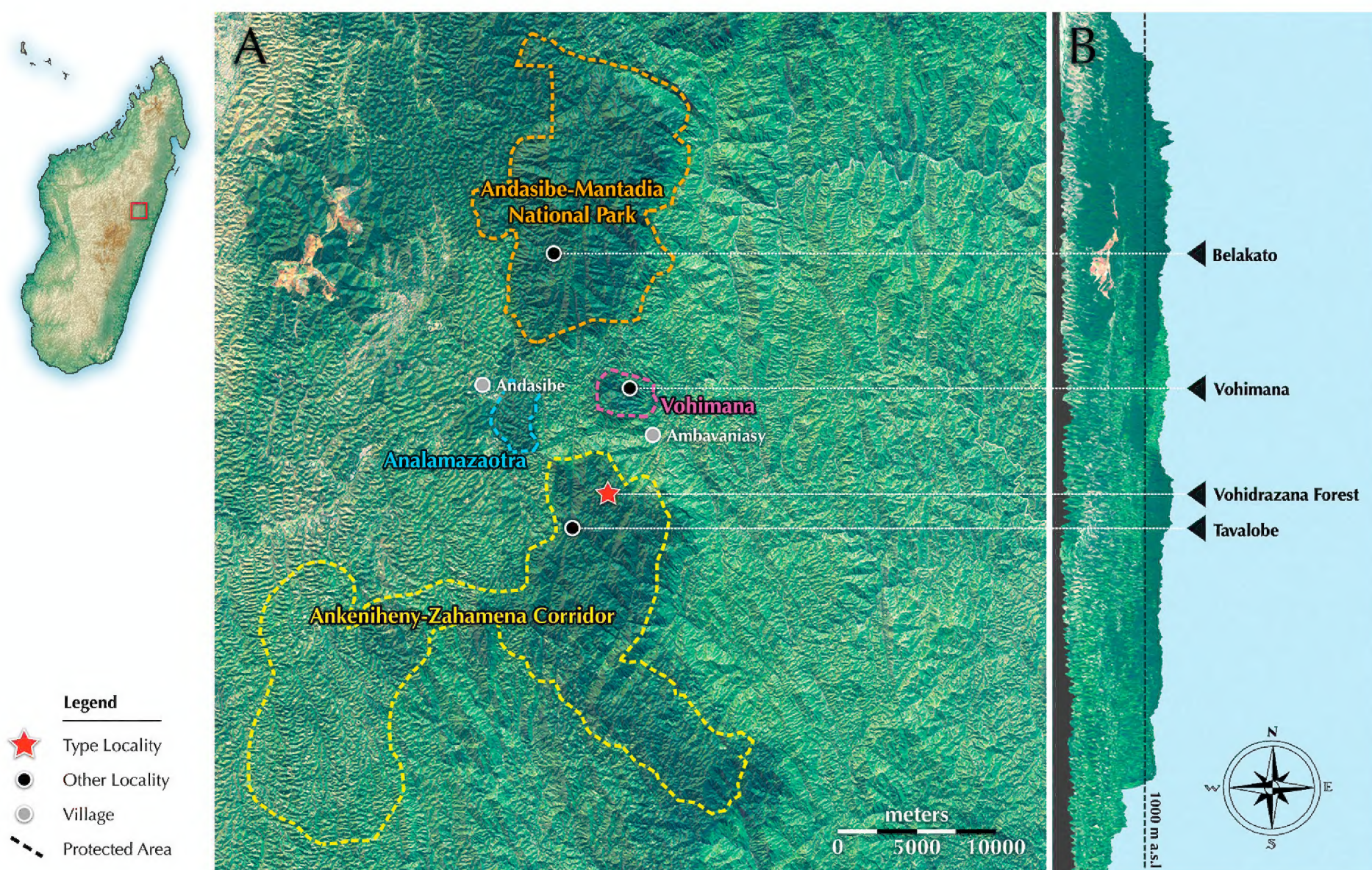
Figure 1. The distribution of *Gephyromantis marokoroko* sp. nov. in east-central Madagascar, view from above (**A.**) and from a profile view (**B.**). The black star marker indicates the type locality at Vohidrazana Forest where the black circle "locality" markers indicate other confirmed localities for the new species. *Gephyromantis marokoroko* sp. nov. is also found at high elevations and, thus, is likely distributed at other high elevation sites not surveyed. Elevational and satellite imagery data acquired from the USGS Earth Explorer (http://earthexplorer.usgs.gov).

the subgenus *Laurentomantis* through its irregular and rough granular dorsum, single subgular vocal sac in males, completely connected lateral metatarsalia, inner and outer metatarsal tubercle present and tympanum is the same size in male and female.

Gephyromantis marokoroko is characterised by bright red eyes, prominent ridge elements on dorsum, life colouration with a dark brown ground colour with mottled red and grey, hind-limbs dark brown containing red crossbands, absence of red colouration on the sides of thighs and ventre, white spots on grey-coloured ventre and males with bulbous type 2 femoral glands with eight granules in two rows of four on each thigh. Furthermore, the new species is characterised by an advertisement call with a moderately long call duration (1095–1431 ms), 22–28 notes/call, 2–4 strong amplitude-modulated pulses per note and a dominant frequency of 2250–2812 Hz. Finally, *Gephyromantis marokoroko* has a large genetic distance of 6% or greater amongst related species in the 16S rRNA marker and has strongly supported reciprocal monophyly to all other species in *Laurentomantis* (Fig. 3).

Gephyromantis marokoroko can be distinguished from other members of *Laurentomantis* morphologically (Table 1; Fig. 5). The rugose and granular dorsal texture with prominent ridge elements and red mottled colouration and the larger number of eight prominent femoral gland granules per femur readily characterise this species from other *Laurentomantis* (Figs 3 and 5). The new species is easily distinguished from *G. horridus* (Boettger
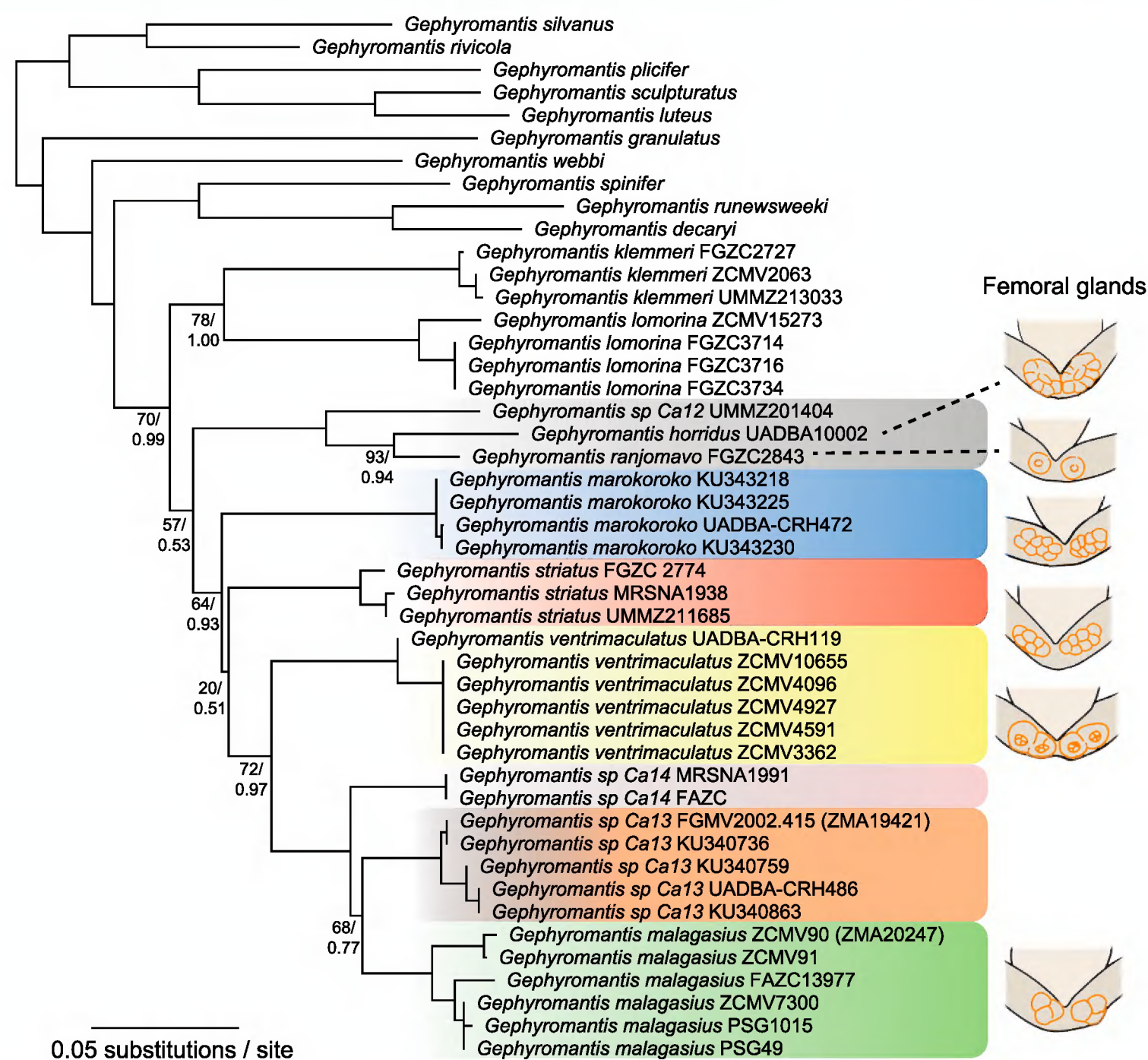
1880), *G. malagasius* (Methuen and Hewitt 1913) and *G. ranjomavo* (Glaw and Vences 2011) by lacking tibial glands, its larger number of femoral gland granules and its rugose and granular dorsal texture with prominent ridge elements. Furthermore, the new species is easily distinguished from *G. ventrimaculatus*, where *G. marokoroko* has eight distinct femoral gland granules on each thigh (eight irregularly-shaped femoral gland granules in *G. ventrimaculatus*), by the dark grey and red dorsal colouration (light brown in *G. ventrimaculatus*) and by lacking blue marbling on the ventral surfaces (Fig. 5). The most similar species morphologically is *G. striatus* (Vences et al. 2002), but the new species differs from *G. striatus* through its larger number of femoral gland granules (8 vs. 3–6), the vertebral stripe is absent or indistinct and short (always distinct in *G. striatus*), bright red eye (orange-brown in *G. striatus*) and its prominent and strong ridge elements, as well as the dark grey and red colouration on the dorsum (weak ridge elements and brown and orange colouration on the dorsum in *G. striatus*).

Bioacoustically, the advertisement call of *Gephyromantis marokoroko* is similar to other species in *Laurentomantis* and can be distinguished from all other species in this subgenus through the following combination of continuous call characters: (1) moderately long call duration (1095–1431 ms); (2) 2–4 strongly amplitude-modulated pulses per note; and (3) a note repetition rate of 14–20 notes/s. *Gephyromantis striatus*, *G. malagasius* and *G. horridus* have overlapping call durations with the new

Figure 2. Ex-situ dorsal-lateral, dorsal and ventral photographs of **A.** Male *Gephyromantis marokoroko* sp. nov. (holotype, KU 343230) and **B.** Female (paratype, KU 343218) in life.

Gephyromantis silvanus
Gephyromantis rivicola
Gephyromantis plicifer
Gephyromantis sculpturatus
Gephyromantis luteus
Gephyromantis granulatus
Gephyromantis webbi
Gephyromantis spinifer
Gephyromantis runewsweeki
Gephyromantis decaryi
Gephyromantis klemmeri FGZC2727
Gephyromantis klemmeri ZCMV2063
Gephyromantis klemmeri UMMZ213033
Gephyromantis lomorina ZCMV15273
Gephyromantis lomorina FGZC3714
Gephyromantis lomorina FGZC3716
Gephyromantis lomorina FGZC3734
Gephyromantis sp Ca12 UMMZ201404
Gephyromantis horridus UADBA10002
Gephyromantis ranjomavo FGZC2843
Gephyromantis marokoroko KU343218
Gephyromantis marokoroko KU343225
Gephyromantis marokoroko UADBA-CRH472
Gephyromantis marokoroko KU343230
Gephyromantis striatus FGZC 2774
Gephyromantis striatus MRSNA1938
Gephyromantis striatus UMMZ211685
Gephyromantis ventrimaculatus UADBA-CRH119
Gephyromantis ventrimaculatus ZCMV10655
Gephyromantis ventrimaculatus ZCMV4096
Gephyromantis ventrimaculatus ZCMV4927
Gephyromantis ventrimaculatus ZCMV4591
Gephyromantis ventrimaculatus ZCMV3362
Gephyromantis sp Ca14 MRSNA1991
Gephyromantis sp Ca14 FAZC
Gephyromantis sp Ca13 FGMV2002.415 (ZMA19421)
Gephyromantis sp Ca13 KU340736
Gephyromantis sp Ca13 KU340759
Gephyromantis sp Ca13 UADBA-CRH486
Gephyromantis sp Ca13 KU340863
Gephyromantis malagasius ZCMV90 (ZMA20247)
Gephyromantis malagasius ZCMV91
Gephyromantis malagasius FAZC13977
Gephyromantis malagasius ZCMV7300
Gephyromantis malagasius PSG1015
Gephyromantis malagasius PSG49

Femoral glands

Node support values: 78/1.00; 70/0.99; 93/0.94; 57/0.53; 64/0.93; 20/0.51; 72/0.97; 68/0.77

0.05 substitutions / site

Figure 3. Results of phylogenetic analysis of the mitochondrial 16S rRNA barcode 3' marker for Maximum Likelihood (ML) and Bayesian Inference (BI). Topology is a consensus tree from IQ-Tree. The support values are shown as Bootstrap on top and Posterior Probability on the bottom only for nodes that were not perfectly supported. Note that *Gephyromantis marokoroko* sp. nov. placement in the clade is weakly supported in both analyses.

Table 1. Comparison of distinguishing characters used to differentiate species within *Laurentomantis*. Table adapted from Vences et al. (2002) combined with new data. Genetic distances are uncorrected and taken from the 16S rRNA mitochondrial marker.

| Character	Species					
	G. marokoroko sp. nov.	*G. striatus*	*G. ventrimaculatus*	*G. horridus*	*G. malagasius*	*G. ranjomavo*
Male SVL (mm)	24.0–27.0	22.2–23.8	23.0–25.0	26.0–28.1	20.2–24.0	23.5–25.8
Female SVL (mm)	23.9–24.6	23.9–26.9	29.1	35.4	23.2–25.7	n/a
Tibial gland	Absent	Absent	Absent	Present	Present	Present
Granules in femoral glands (per femur)	8	3–6	9	5–6	1–4	1
Dorsal skin texture	Strongly granular; strong ridge elements	Moderately granular; weak ridge elements	Strongly granular; strong ridge elements	Strongly granular; weak ridge elements	Strongly granular; weak ridge elements	Moderately granular, weak ridge elements
Ventral skin texture	Slightly granular	Smooth	Smooth	Granular	Slightly granular	Smooth
Red colour on hind-limbs	Absent	Absent	Absent	Absent	Present	Absent
Dorsal colour	Dark grey with bright red marbling	Dark grey with brown and orange marbling	Dark grey with brown marbling	Dark grey with brown marbling	Brown with lighter brown marbling	Brown with yellow mottling, orange limbs
Ventral colour	Dark-grey brown with light spotting	Dark grey-brown with few light spots	Brown with blue marbling	Dark with grey marbling	Brown with light marbling	Light brown with yellow, light spots
Vertebral stripe	Absent or indistinct	Present	Absent	Absent or indistinct	Absent	Absent
Advertisement call duration (ms)	1095–1431	440–1266	407–455	1271–2521	768–1468	n/a
Advertisement call note repetition rate /s	14–20	29–40	21–24	13	18–36	n/a
Genetic distance (from *G. marokoroko*)	0.25–1.5%	7–9%	6–9%	10–11%	8–11%	12%

species and overlapping note repetition rates, except for *G. striatus*, which has the fastest note repetition rate (Table 1). Despite these similarities, the clearly pulsed notes alone distinguish the new species from all other *Laurentomantis*, except *G. ventrimaculatus* (Angel 1935), which has ca. 5–6 pulses/note; however, *G. ventrimaculatus* differs by having the shortest call duration non-overlapping with other *Laurentomantis* species at 407–455 ms and a slightly faster note repetition rate of 21–24 notes/s. Temperature is not likely to be an important factor in the characteristic differences described here, as structural characters, such as clearly defined pulses, would not be affected by temperature (Schneider 1974).

Motivation might affect number of notes emitted and, thus, call duration; however, the recording of *G. ventrimaculatus* is of a highly motivated male (i.e. many calls emitted in a short time) while the call of the new species was recorded from males which did not appear to be very motivated, emitting only 1–2 calls within an hour. Finally, comparisons could not be made to *G. ranjomavo* as calls were not available; however, the new species is clearly morphologically distinct (see above).

Description of the holotype. Fixed in 10% buffered formalin solution, preserved in 70% ethanol, in good state of preservation, except for skin loss near the anterior dorsum, with left thigh muscle removed for tissue sample. Adult male, SVL 26.0 mm. Body very slender; head longer than wide HL 33.4% of SVL; slightly wider than body, HW 33.7% of SVL; snout of moderate length, ESD 16.2% of SVL; snout rounded in dorsal and later-al view; nostrils directed laterally, slightly protuberant, nearer to snout tip than eye; ED larger than END; canthus rostralis indistinct, concave; loreal region slightly concave; single subgular vocal sac; gular glands absent. Tympanic annulus distinct and round, small, TD 64.5% of ED; supratympanic fold indistinct and irregular, tympanic membrane lighter than ground colouration. Vomerine teeth not visible on the buccal roof, present under mucosal skin; choanae small, rounded. Tongue longer than wide; ovoid in shape, posteriorly bifid. Dermal fold along lower jaw absent. Arms slender, subarticular tubercles single; outer and inner metacarpal tubercles present, indistinct. Fingers without webbing; nuptial pads absent; relative finger length 2 < 1 < 4 < 3; second finger distinctly shorter than fourth finger, only slightly shorter than finger one; finger discs distinctly enlarged, larger on third and fourth finger. Hind limbs slender; lateral metatarsalia connected; inner metatarsal tubercle distinct, outer metatarsal tubercle small, but recognisable; TIBL 55.2% of SVL; FOL 45.2% of SVL. Tibial glands absent. Toes without webbing; relative toe length 1 < 2 < 5 < 3 < 4; toe three distinctly longer and broader than toe five; toe discs distinctly enlarged. Femoral glands large, well delimited, having eight distinct clusters on each femur of almost the same size, in two rows of four. Skin coarsely granular and heavily rugose on dorsal surfaces; large and sharply elevated tubercles and ridges irregularly distributed across dorsal surfaces, with less distinct ridges on the lower back; some ridges are symmetrical, larger tubercles and short ridges present on
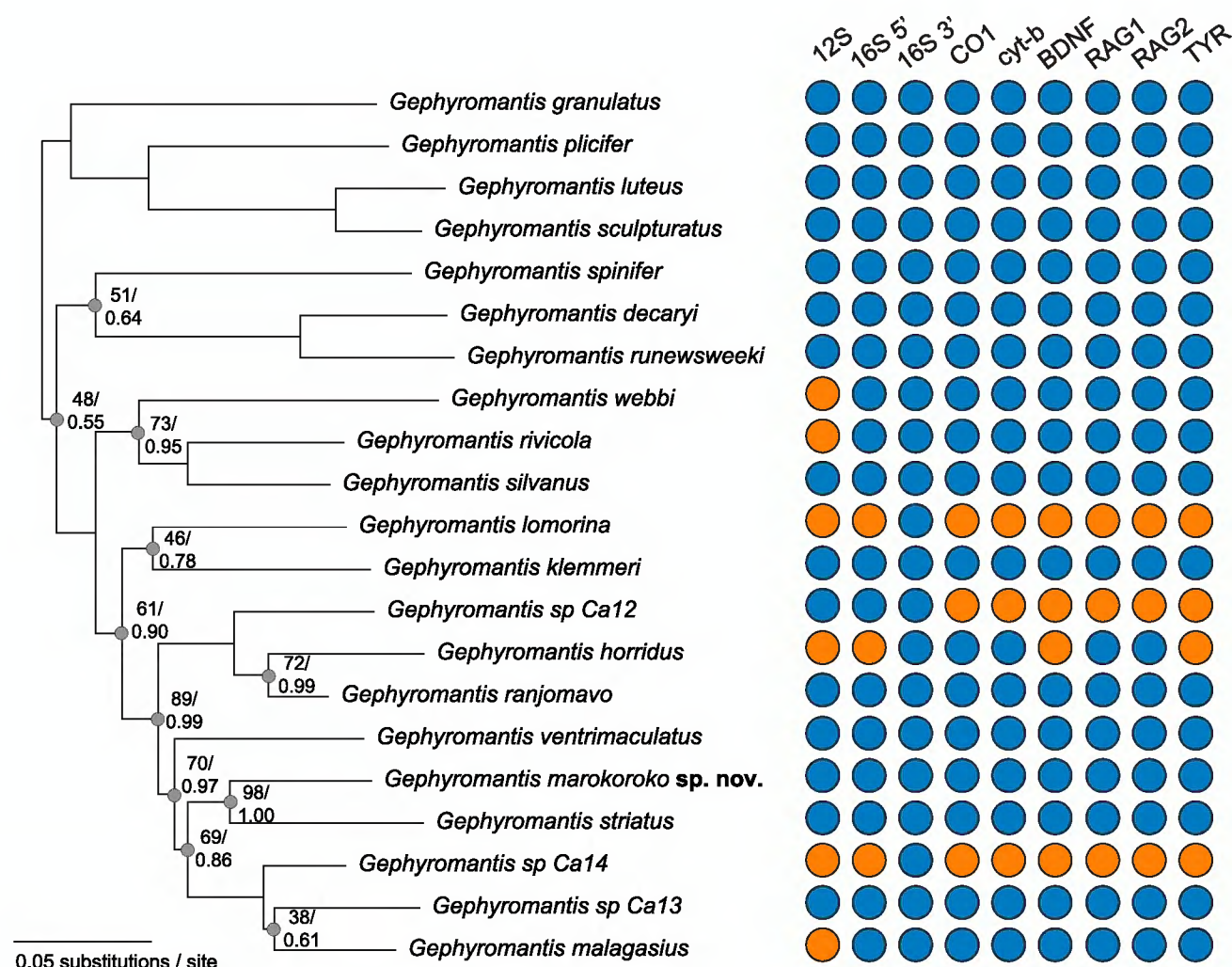
Figure 4. Results of phylogenetic analyses of the concatenated alignment of five mitochondrial and four nuclear markers for Maximum Likelihood (ML) and Bayesian Inference (BI). Topology is a consensus tree from IQ-Tree. On the right, the dots represent markers that were present in blue and absent in orange for each sample. The notes marked with a circle are those that did not receive perfect support (Bootstrap = 100; Posterior Probability = 1.00) from ML and BI, with the support values as BS on top and PP on the bottom. Note that *Gephyromantis marokoroko* sp. nov. has strong support in both analyses for a sister relationship to *G. striatus*.

Figure 5. Ex-situ dorsal-lateral, dorsal and ventral photographs of **A.** Male *Gephyromantis marokoroko* sp. nov. (holotype, KU 343230); **B.** *Gephyromantis striatus* (Marojejy, ZCMV 15140; photographs by Mark D. Scherz); and **C.** *Gephyromantis ventrimaculatus* (Ranomanfana, KU 340917).

head and anterior dorsal region. Ventral skin granular on stomach, throat and limbs.

After four years in preservative, dorsal ground colouration is a uniform dull brown including forelimbs and hind-limbs. The red colouration has faded to become light brown. Lighter coloured spots on ventral surfaces are still present.

In life (Fig. 2), dorsal colouration is a dark grey ground colour with thick, bright red mottling distributed on the dorsum. Many of the raised ridges are dark grey with bright red edges. Lighter red stripe present short distance up the dorsum. Lateral head the same as dorsum, tympanum a lighter brown. Flanks are also dark grey, but have less bright red colouration, typically only found on ridges. Forelimbs have same colouration as dorsum, except bright red colouration is more spotted, with a few lighter

red spots. Hind-limbs have same colouration as forelimbs, except with red crossbands present on the dorsal surface. A whitish annulus is present before the terminal disc on fingers and toes, fingers and toes light brown. Ventral surfaces brown, with no red present. White and light-yellow spots are present and scattered moderately along the ventre. Ventral sides of arms and hind-limbs brownish-grey, with light red spotting. Femoral glands lighter brown than surrounding limb surfaces. Single subgular vocal sac is light grey, with some light-yellow spotting down the centre. Jaw has scattered light-red spots along the lip. The pupil is black with a bright red iris, with black reticulations around the outer margin of the iris.

Variation. All paratypes resemble the holotype in morphology and colouration. In life, dorsal colouration varies slightly in the amount and intensity of red present.

Spotting on the ventral surfaces varies in the colouration of the spots being white, light-yellow, light-orange or light-red. The vertebral stripe varies from being absent in some individuals to indistinct in others. Females lack femoral glands and have a granular texture on the femur.

Morphometry of type series. Measurements of the holotype and paratypes are shown in Table 2.

Bioacoustics. We recorded three calls from two males at Vohidrazana Forest after collection at ca. 02:00 hr on 6 January 2016. Males call infrequently with extremely quiet calls from the upper surfaces of leaves up to 50 cm above the ground. The recorded male was captured and placed in a separate plastic collecting bag. Males would not call when we were within recording distance, so we placed the microphone 100 cm away from the bag near where it was captured and moved several metres away. Calls were recorded during light rain at a temperature of 20.4 °C.

The advertisement call of this species sounds like a heavily pulsed trill or 'groan' to the human observer, emitted irregularly. We define each groan as a call (Fig. 6A–C) with a duration of 1095.1–1431.9 (1221.5 ± 183.5; $n = 3$) ms. Each call consisted of a series of 22–28 (24 ± 3.46; $n = 72$) short notes with a duration of 12–29 (19.9 ± 4.3; $n = 72$) ms and an inter-note duration of 15.5–43.7 (32.3 ± 5.6; $n = 71$) ms. Note rate within each call was 14.4–20.1 (17.9 ± 3.1; $n = 3$) note/s. Each note was strongly pulsed, with 2–4 (3.1 ± 0.783; $n = 72$) pulses per note and a pulse rate of 111.1–235.3 (156.3 ± 25.8) pulses/s (Fig. 6D–F). The call was strongly amplitude-modulated, beginning at a lower amplitude and increasing to the middle of the call, where the amplitude then decreased until the end of the call. The dominant frequency measured at peak amplitude of the call was 2390–2672 (2483 ± 162; $n = 3$) Hz, while the dominant frequency at the peak amplitude of the note was 2250–2813 (2458 ± 149; $n = 72$) Hz. For notes, the 90% bandwidth was from 1453–2297 (1942 ± 177; $n = 72$) Hz to 3000–4125 (3749 ± 290; $n = 72$) Hz. No harmonic frequencies were visible on the spectrogram (Fig. 6).

Phylogenetics. The phylogenetic results support the morphological diagnosis by placing *Gephyromantis marokoroko* within the *Laurentomantis* subgenus with strong support. At the species level, *G. marokoroko* is monophyletic with strong support in ML and BI analyses (BS = 100, PP = 1.00; Fig. 3). Uncorrected p-distances, using the 16S fragment, indicate that *G. ventrimaculatus* has the lowest distance to the new species, at ~ 6–9%. The combined nine marker multi-locus dataset places the new species sister to *G. striatus* with strong support (BS = 98; PP = 1.00) in both BI and ML analyses (Fig. 4). Overall, these results provide strong evidence that the species is a separately evolving lineage and strong evidence for the new species phylogenetic placement.

Distribution. *Gephyromantis marokoroko* is known from several sites in the forests in the vicinity of Andasibe, but has only been found at high elevation sites (~

Table 2. Morphometric measurements (in mm) of the holotype and paratypes of *Gephyromantis marokoroko* sp. nov. Femoral Gland Clusters (FGC) shown as "left, right" count.

Type status	Specimen					
	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype
Field Number	CRH 1110	CRH 1108	CRH 1397	CRH 1061	CRH 1923	CRH 2019
Museum Number	KU 343230	KU 343229	KU 343218	UADBA CRH1061	KU 347328	KU 347329
Sex	M	M	M	M	F	F
SVL	26.0	25.3	27.0	24.0	24.6	23.9
HW	8.8	9.4	8.6	8.6	8.4	8.6
HL	8.7	10.0	8.8	8.2	8.7	8.6
ED	3.8	3.6	3.5	3.8	3.6	3.9
IOD	2.5	2.7	2.7	2.5	2.8	2.5
ESD	4.2	4.0	4.4	4.4	4.1	4.3
END	3.0	3.5	3.3	3.1	2.3	2.9
NSD	1.2	1.5	1.7	1.5	1.9	1.4
NND	2.4	2.6	2.5	2.3	2.4	2.2
TD	2.5	1.6	2.4	2.5	2.0	2.2
FIL	5.1	14.9	6.3	6.2	4.9	5.0
FIIL	6.8	5.7	8.1	8.2	6.4	6.6
FEML	13.9	14.1	14.3	13.4	14.1	14.1
TIBL	14.4	14.3	14.4	13.3	14.6	13.8
FOL	11.8	11.3	12.2	11.2	12.1	11.3
TARL	8.1	8.2	8.4	7.6	8.4	8.0
HAL	8.4	8.3	7.9	8.2	8.3	7.6
LAL	7.7	7.4	8.3	7.4	8.1	8.2
UAL	6.1	6.2	6.2	6.0	6.2	6.0
FORL	22.2	21.8	22.3	21.6	22.6	21.7
FGL	6.4	5.8	10.1	5.7	-	-
FGW	2.8	2.4	3.3	3.2	-	-
FGC	8; 8	8; 8	8; 8	8; 8	-	-
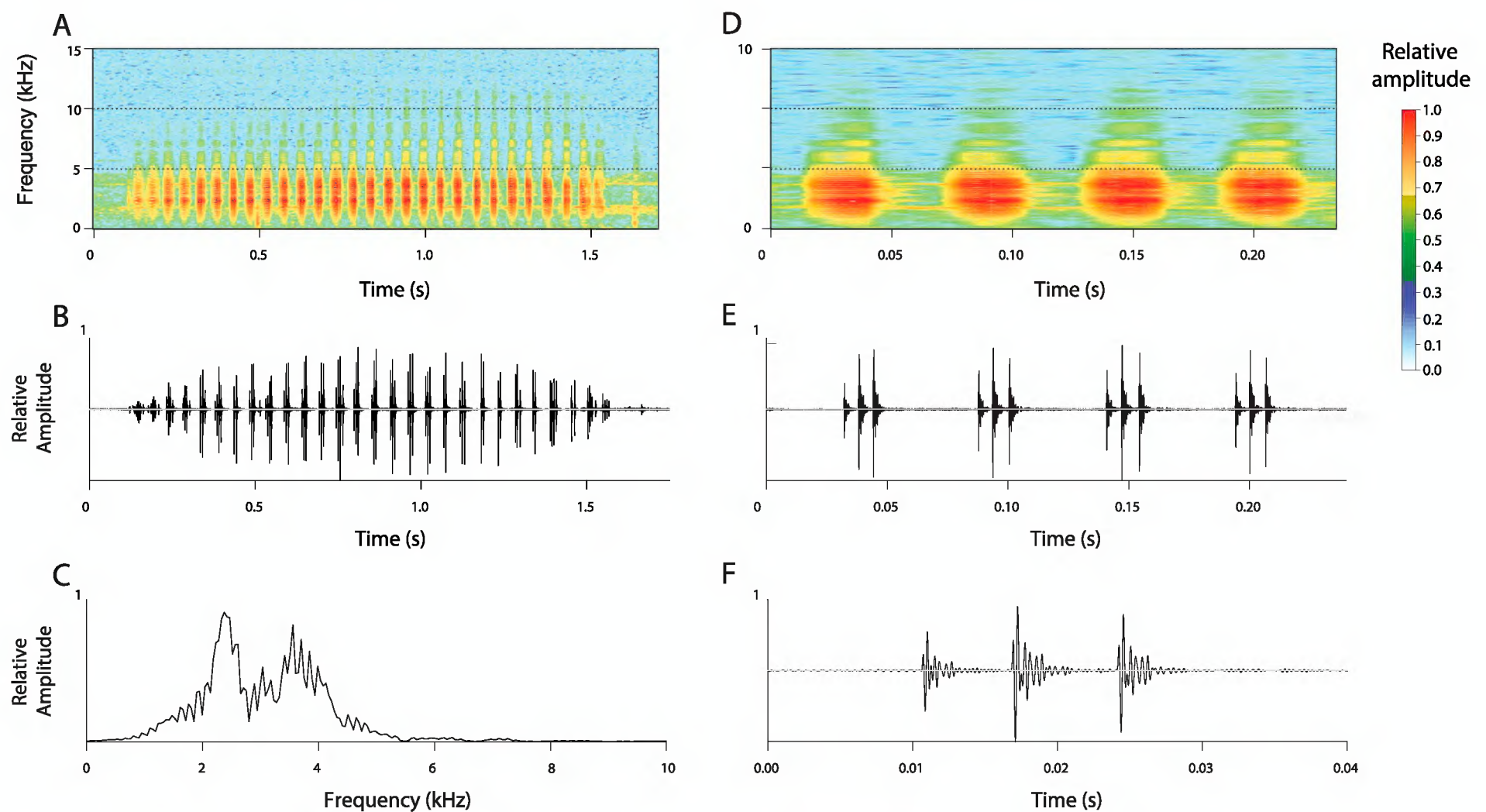
Figure 6. Oscillograms and spectrograms of the call of *Gephyromantis marokoroko* sp. nov. (Holotype: KU 343230). **A.** The entire call spectrogram and **B.** Entire call oscillogram; **C.** Power spectra/frequency spectrogram of a single note; **D.** A close-up spectrogram of four notes and **E.** Corresponding oscillogram; and **F.** an individual note taken from the middle of the call.

1000–1200 m a.s.l.; Fig. 1). The new species is known from the following localities: Vohidrazana Forest (18.976°S, 48.499°E), Tavalobe (19.005°S, 48.461°E), Vohimana (18.926°S, 48.489°E) and Andasibe-Mantadia National Park (Belakato: 18.821°S, 48.439°E).

Natural history. *Gephyromantis marokoroko* is apparently locally rare and, thus far, only found within undisturbed, primary forests at highland elevations (ca. 1000–1200 m). Individuals of the species were perched on the surfaces of vegetation less than 50 cm in height (Fig. 7). The species was infrequently encountered, always after moderate to heavy rain, with multiple individuals occasionally grouped in small clusters (~ 20 m²). The species' call is very quiet and irregular and is barely audible to a human observer, even within three metres of a calling individual. Individuals of the new species were often found syntopically with another *Laurentomantis*, *G.* sp. Ca13, which is a candidate species identified in Vieites et al. (2009). Other syntopic *Gephyromantis* include *G. eiselti*, *G. salegy*, *G.* sp. aff. *plicifer* (not yet assessed for a candidate species number) and *G. cornutus*.

Conservation status. The new species is known from Andasibe-Mantadia National Park and several other managed areas (e.g. Vohimana, the community managed Vohidrazana Forest and Tavalobe). However, as currently understood, the distribution of this species is severely fragmented and restricted to only four known high-elevation localities (~ 1000–1200 m), which are very small patches with no connectivity (Fig. 1). Many other high elevation sites in the region have been surveyed by the authors over three field seasons. Furthermore, Vohidra-

zana Forest and Tavalobe face ongoing threats that result in the reduction of quality and extent of habitat. For example, slash-and-burn agriculture and forest products are frequently extracted directly from this species' habitats that are outside protected areas. Given this information, we categorise this species as "Endangered" [B1ab(iii-iv)] following IUCN Criteria (IUCN 2001).

Discussion

Gephyromantis (*Laurentomantis*) *marokoroko* sp. nov. is a clearly distinct species, as evidenced through morphology, bioacoustics and molecular phylogenetics. The new species can be readily distinguished from other members in *Laurentomantis* by its heavily rugose granular skin, vibrant red eyes, bright red body colouration and distinctive femoral glands. The call of *G. marokoroko* also differs from all other *Laurentomantis* through its moderately long call duration, clearly pulsed notes and slower note repetition rate. Phylogenetic analyses strongly support the new species as monophyletic in the 16S rRNA mitochondrial marker multi-sample dataset. Additionally, the single-sample per species dataset of nine-markers (five mitochondrial and four nuclear) and both phylogenetic analyses strongly supported *G. striatus* and *G. marokoroko* as sister species (Fig. 4). In addition, morphological similarity in the number and shape of femoral glands and the occasional presence of vertebral stripe support this relationship.

Gephyromantis marokoroko is a remarkable discovery that was immediately obvious as a new species in the field
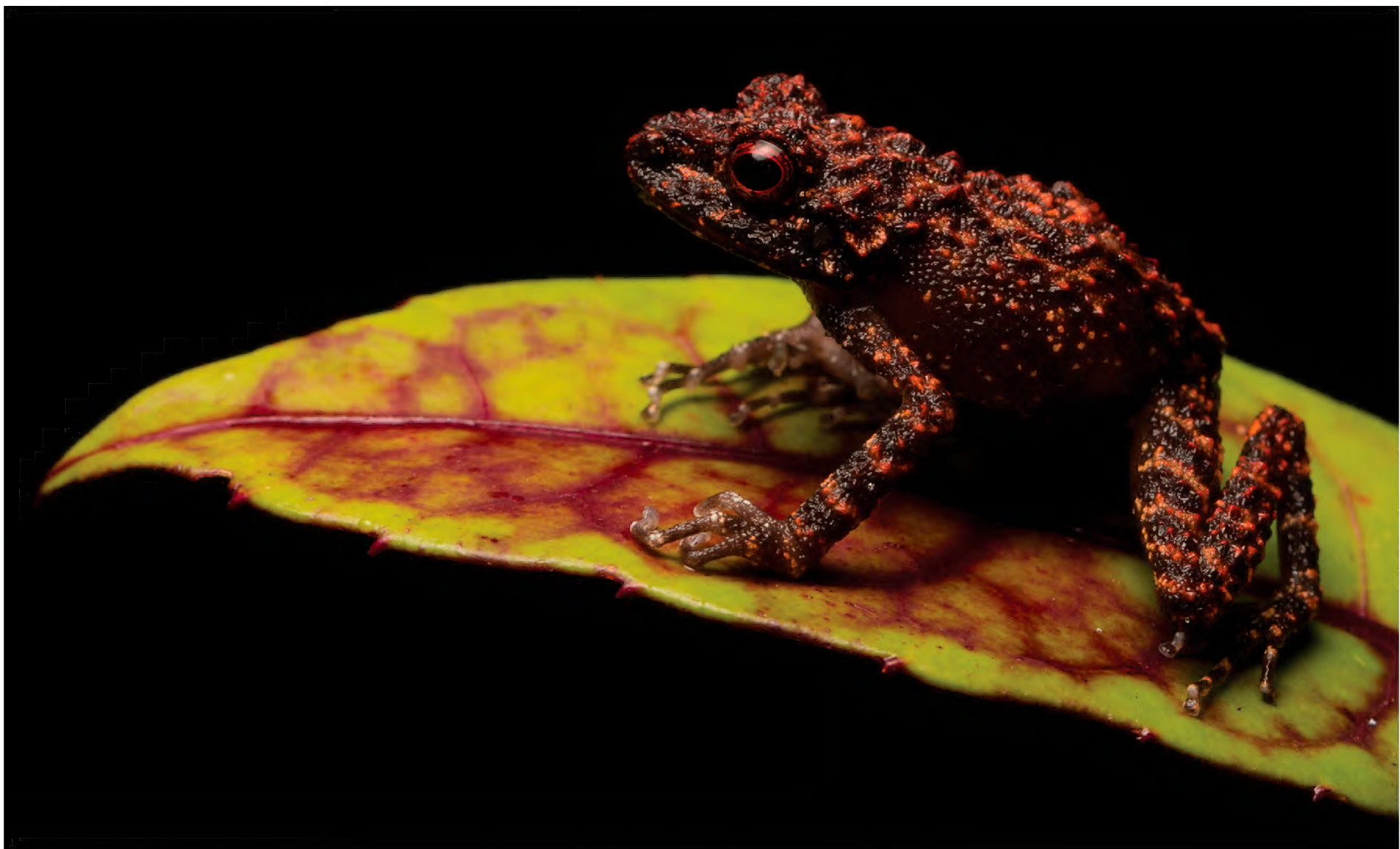
Figure 7. In-situ photograph of *Gephyromantis marokoroko* sp. nov. (UADBA-CRH1629).

as its general appearance is very distinct and spectacular, with several clear morphological differences from related species. Distinctive new species are typically discovered in poorly unexplored areas and *G. marokoroko* was discovered in the well-explored vicinity of Andasibe. In addition, the species had never been barcoded before, eluding past herpetological surveys. This new species highlights the importance of continued fieldwork in Madagascar, as the discovery of previously undocumented new species is occurring frequently (Lambert et al. 2017; Scherz et al. 2017a; Scherz et al. 2018), despite the extensive past barcoding efforts for Malagasy frogs (e.g. Vieites et al. 2009; Perl et al. 2014). Typically, such species have low population densities, small geographic or elevational ranges and/or are in areas that have not been extensively surveyed. Many recent species descriptions of Malagasy frogs are from previously-known candidate species and/or are cryptic lineages that required molecular evidence to diagnose (e.g. Hutter et al. 2015; Scherz et al. 2017c; Vences et al. 2017) and it is uncommon to find new species that have not already been documented through barcoding efforts (e.g. Vieites et al. 2009; Perl et al. 2014). These discoveries are rare and there are only a few recent examples of new discoveries that include *G. lomorina* (Scherz et al. 2018) and *Boophis masoala* (Glaw et al. 2018). The continuation of basic field inventories is, therefore, clearly necessary to fully understand the patterns of species richness and complete evolutionary histories of frogs in Madagascar and other tropical regions.

The discovery and conservation of these new and unique species is critically important as habitat loss continues, especially in the study area. The distribution of *G. marokoroko* is severely fragmented and restricted to only four locations and occurs in small habitat patches (Fig. 1). While the species is protected within Andasibe-Mantadia National Park and Vohimana Special Reserve, it has only been found in low abundance in single, very small habitat patches. The localities Vohidrazana Forest and Tavalobe face ongoing threats from slash-and-burn agriculture and forest products are frequently extracted directly from this species' habitat that are outside protected areas. Furthermore, climate change could exacerbate these risks reducing further the suitable habitat for this already micro-endemic species.

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Author contributions

CRH and SML conceived the study. CRH wrote the first draft of the manuscript and the other authors provided input. ZFA collected phenotypic data. All the co-authors were involved with fieldwork, data collection and logistics.

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